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## Alterations in Rat Aortic $\alpha_1$ -Adrenoceptors and $\alpha_1$ -Adrenergic Stimulated Phosphoinositide Hydrolysis in Intraperitoneal Sepsis

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We investigated the alterations of rat aortic  $\alpha_1$ -adrenoceptors and  $\alpha_1$ -adrenergic stimulated phosphoinositide (PI) metabolism in intraperitoneal sepsis. An analysis of [ $^{125}$ I]-hydroxyethylaminotetralone (HEAT) binding to  $\alpha_1$ -adrenoceptors on rat aortic membranes revealed decreased numbers of receptors without changes in affinity. The maximum number of binding sites decreased from  $349 \pm 35$  fmol/mg to  $146 \pm 16$  fmol/mg ( $P < 0.05$  vs. control). PI metabolism was similarly attenuated in aortae from septic rats. The norepinephrine-stimulated hydrolysis of [ $^{32}$ P]-phosphatidylinositol-4,5-bisphosphate was significantly decreased in aortae from septic rats as was the  $\alpha_1$ -adrenoceptor stimulated accumulation of [ $^3$ H]-inositol monophosphate. Finally, the basal labeling of [ $^{32}$ P]-phosphatidylinositol-4,5-bisphosphate but not of [ $^{32}$ P]-phosphatidylinositol or [ $^{32}$ P]-phosphatidic acid was significantly diminished. These results imply that signal transduction induced by  $\alpha_1$ -adrenoceptor agonists in rat aorta is significantly altered in intraperitoneal sepsis. These findings may help define the mechanisms of depressed aortic contractility in models of sepsis and endotoxic shock.

**Key words:** septic shock, phosphoinositide metabolism, vascular contraction, vasoconstriction, signal transduction

### INTRODUCTION

Sepsis and septic shock are major causes of death in the United States among critically ill patients. Despite sophisticated and aggressive surgical and medical

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A preliminary account of these findings was presented at the first International Shock Conference and published in abstract form [36].

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interventions, the mortality rate in septic shock remains between 40 and 60% [1]. In dissecting the pathophysiology of sepsis and endotoxemia, numerous investigators have noted diminished peripheral vascular responsiveness to norepinephrine in both humans [2] and in animal models of sepsis and endotoxemia [3,4]. Furthermore, an attenuated response to exogenously applied norepinephrine (NE) has been noted in isolated aorta by several groups of investigators [5,6] using a variety of models of sepsis and endotoxemia. The mechanism of this phenomenon remains unknown [see refs. 3,4 for review], but could reside in part in the signal transduction apparatus.

We recently found that NE-induced contraction of rat aorta, which is mediated by  $\alpha_1$ -adrenoceptors, appears to correlate with the breakdown of phosphoinositides (PI) [7]. Our original observations have now been replicated [8]. We also noted that the potent vasoconstrictor 5-hydroxytryptamine [9] and several vasoactive prostaglandins [10] also appear to induce rat aortic contraction, at least in part, via PI breakdown [for review see refs. 3,4]. According to this scheme, following binding of a ligand to the receptor a phosphoinositide-specific phospholipase C is activated which cleaves phosphatidylinositol-4,5-bisphosphate to release inositol-triphosphate ( $IP_3$ ) as well as diacylglycerol (DAG). It is proposed that  $IP_3$  mobilizes intracellular calcium [11-15] while DAG activates protein kinase C. Phorbol esters, which mimic the effects of endogenous DAG, are potent inducers of rat aortic contraction. Phorbol esters induce vasoconstriction in part by the mobilization of extracellular calcium in a nitrendipine-sensitive fashion [16].

We previously discovered that hepatic  $\alpha_1$ -adrenoceptors [17], as well as vasopressin receptors [18], were decreased in intraperitoneal sepsis as well as in chronic endotoxin infusion models of sepsis [19]. Spitzer and colleagues [20] found that the  $\alpha_1$ -adrenoceptors and vasopressin receptor-mediated mobilization of intracellular calcium and activation of phosphoinositide hydrolysis were altered in intraperitoneal sepsis and in endotoxemia. These findings suggested to us that vascular PI metabolism and adrenoceptors might be similarly altered in intraperitoneal sepsis.

In this paper we report significant alterations in aortic PI metabolism in rat intraperitoneal sepsis. We also discovered diminution of aortic  $\alpha_1$ -adrenoceptors. These results suggest that experimental sepsis in the rat induces alterations in receptor-coupled signal transduction in the aorta.

## MATERIALS AND METHODS

### Animals and Their Treatment

Sprague-Dawley rats (Taconic Farms, Baltimore, MD) weighing 200-350 g were used in all experiments. Cecal ligation with two-hole puncture (CLP) was performed as previously detailed [17]. The sham procedure was identical except that the cecum was not devascularized, ligated or punctured. At 18-24 hr post-procedure, surviving animals (60-70% survival at this time point) were sacrificed by decapitation. Surviving animals displayed the signs of sepsis described by Wichterman et al. [21] including piloerection, a bloody discharge from the nose and mucous membranes, bloody diarrhea and lethargy.

### Phosphoinositide Metabolism

PI turnover in the rat aorta measuring [ $^3H$ ]-inositol monophosphate accumulation in the presence of 10 mM LiCl was determined by a modification of the

procedures of Berridge et al. [22] as previously described [7,9,23,24] using [<sup>3</sup>H]-myo-inositol (16 Ci/mmol, New England Nuclear, Boston, MA). We previously showed that this procedure accurately separates inositol mono-, bis- and tris-phosphates.

For measurement of [<sup>32</sup>P]-phosphoinositide metabolism, rat aortic rings (4 mm length) were prepared [10] and pre-incubated for 15 min in an oxygenated Hepes buffer of the following composition (in mM) at 37°C: 140 NaCl, 10 D-glucose, 5 Hepes, 1.0 MgCl<sub>2</sub>, 1.5 CaCl<sub>2</sub> pH-7.40. The segments were then incubated in [<sup>32</sup>P]-orthophosphate (carrier free, Amersham) containing Hepes buffer (30 μCi/ml) for 30 min to label phosphoinositide pools. Preliminary experiments disclosed steady-state labeling of PI pools by this time period. Test agents were then added for various periods of time and the reaction terminated by the addition of 0.9 ml chloroform/methanol/HCl (100:200:0.1) solution followed by the addition of 0.3 ml water and 0.3 ml chloroform. Following lipid extraction, the lower phase was removed, washed twice with upper phase and concentrated in a Speed-Vac. The samples were then applied to oxalate pre-coated high performance thin layer chromatography plates (HPTLC) prepared and run according to Jolles et al. [25]. [<sup>32</sup>P]-phosphoinositides were identified by autoradiography with Kodak X-OMAT film (XAR-2) and by authentic standards (Sigma Chemical Co., St. Louis, MO). The spots so identified were scraped into scintillation vials and quantified by liquid scintillation spectrometry.

#### Alpha<sub>1</sub>-Adrenoceptor Measurements in Rat Aorta

Aortas from 12 rats (sham or CLP) were homogenized in 20 mM Tris-Cl buffer (pH-7.40, 25°C) with a polytron homogenizer and then rehomogenized with a tightly-fitting glass-glass homogenizer. This homogenate was centrifuged at 1,000 × g for 15 min (4°C) to sediment cellular debris and then a crude plasma membrane fraction was prepared by centrifugation at 35,000 × g for 45 min. The resulting pellet was resuspended in binding buffer [17] and incubated (0.5 ml t.v.) at 25°C for 60 min in the presence of increasing doses of the selective alpha<sub>1</sub>-agonist [<sup>125</sup>I]-hydroxyethylaminotetralone (HEAT) (2,200 Ci/mmol, New England Nuclear, Boston, MA) in the presence and absence of 1 μM prazosin to determine specific binding. Membranes were harvested by filtration on GF/B filters (Whatman) and washed by three 5-ml washes of ice-cold binding buffer. Filters were placed into vials and counted in a gamma counter.

Binding data were analyzed and binding parameters (K<sub>d</sub>, B<sub>max</sub>) determined using the nonlinear least-squares computerized curve fitting program (LIGAND) as previously detailed [26] using the NIH DEC/10 computer. This iterative procedure constructs models of binding according to the law of mass action for the interaction of multiple ligands with multiple binding sites. The results of three or more experiments were averaged to provide a weighted mean and SEM. Protein concentration was determined as described by Bradford [27].

#### Materials

Solvents were of reagent grade or better (Fisher Chemical Co., St. Louis, MO); all other materials, unless otherwise specified, were from Sigma Chemical Co. (St. Louis, MO).

## RESULTS

## Alterations in Rat Aortic PI Metabolism

Recent findings [20] have suggested that alterations might exist in hepatic PI metabolism in various sepsis and endotoxin models. We wished to determine whether similar changes might occur in aorta. Figure 1 shows that the NE-activated PI hydrolysis, as measured by the accumulation of [ $^3$ H]-inositol monophosphate, (PI) was significantly attenuated in the aortas from septic rats, when compared with sham-operated controls. Dose-response studies for a short time of incubation have disclosed an  $EC_{50}$  of  $0.93 \pm 0.6 \mu\text{M}$  for NE-induced [ $^3$ H]-IP accumulation [see ref. 21]; prazosin, a selective  $\alpha_1$ -antagonist inhibits the response with an  $IC_{50}$  of 3nM, while yohimbine (an  $\alpha_2$ -antagonist) has an  $IC_{50}$  of 100 nM [7]. Thus, the measurement of NE-induced [ $^3$ H]-IP accumulation in rat aorta accurately reflects the activity of  $\alpha_1$ -adrenoceptors.

Since phosphatidylinositol-4,5-bisphosphate ( $PIP_2$ ) is thought to be the primary substrate for PI hydrolysis in rat aorta [28], we sought to measure the accumulation of [ $^3$ H]-inositol triphosphate ( $IP_3$ ) using our previously described technique. Because of the very small amounts of  $IP_3$  released which are not degraded into IP we were unable to measure this metabolite (not shown). We were, however, able to measure the NE-stimulated breakdown of [ $^{32}$ P]-phosphatidylinositol-4,5-bisphosphate. After a 30 sec exposure to  $10 \mu\text{M}$  NE, [ $^{32}$ P]- $PIP_2$  levels were decreased in aortas from sham-operated rats, but unchanged in aortas from septic rats (Table I). After a 1 min exposure to  $10 \mu\text{M}$  NE [ $^{32}$ P]- $PIP_2$  levels returned to baseline (Table II). This effect is similar to that reported by Rapoport in rat aorta [29].

Because the basal levels of [ $^{32}$ P]- $PIP_2$  as well as basal [ $^3$ H]-IP accumulation were diminished, we sought to determine whether these alterations represented generalized changes in PI synthesis. Accordingly, we measured the labeling of various PI metabolites in aortas from septic and sham-operated rats (Table II) in the basal state

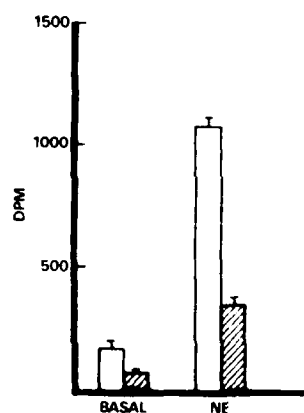


Fig. 1. The effect of intraperitoneal sepsis on norepinephrine-stimulated phosphoinositide hydrolysis in rat aorta. Aortic rings from control (open bars) and septic (hatched bars) were incubated in the presence and absence of  $10 \mu\text{M}$  norepinephrine and [ $^3$ H]-inositol monophosphate accumulation determined. Data represent mean  $\pm$  SEM for 16 individual determinations. The differences in basal and stimulated accumulation are significant ( $P < 0.01$ ).

TABLE I. Altered NE Induced PIP<sub>2</sub> Hydrolysis in Sepsis

	Sham	Septic
Control aorta	700 ± 76 dpm	438 ± 89 dpm <sup>a</sup>
30 sec 10 M NE	567 ± 79 dpm	413 ± 39 dpm <sup>b</sup>

<sup>a</sup>(*P* < 0.05) vs. control in sham operated rats.<sup>b</sup>(*P* > 0.05) or no significant change vs. control in septic rats.TABLE II. Time Course [<sup>32</sup>P] Incorporation Into Phosphoinositides in the Presence of 10 M NE (dpm/mg) in Rat Aorta

	Sham	
	0 min	1 min
PI	311 ± 510	326 ± 95
PIP	159 ± 56	187 ± 69
PIP <sub>2</sub>	294 ± 130	362 ± 160
PA	202 ± 61	295 ± 93
	Septic	
	0 min	1 min
PI	311 ± 75	471 ± 182
PIP	110 ± 51	186 ± 40
PIP <sub>2</sub>	152 ± 53 <sup>a</sup>	157 ± 38 <sup>a</sup>
PA	206 ± 27	523 ± 284

<sup>a</sup>*P* < 0.05 vs. sham. Data represent mean ± SEM of six individual determinations.

as well as after a 1 min exposure 10 μM NE. Decreased PIP<sub>2</sub> labeling continues at the 1 min time points as well as at the 30 sec time point in septic aorta.

As is seen (Fig. 2) there was apparently a selective decrease in [<sup>32</sup>P]-PIP<sub>2</sub> labeling in aortas from septic rats, without changes in the basal levels of [P]-PIP, [<sup>32</sup>P]-PI or [<sup>32</sup>P]-PA. Since PIP<sub>2</sub> has recently been shown to be the preferential substrate for the guanine nucleotide activated phospholipase C in rat aorta [28], this decreased substrate availability could account, in part, for the observed decrease in [<sup>3</sup>H]-IP accumulation. It does not account for the diminished [<sup>32</sup>P]-PIP<sub>2</sub> breakdown. These results suggested to us that earlier events in the signal transduction pathway for aortic alpha<sub>1</sub>-adrenoceptors might be perturbed in intraperitoneal sepsis.

#### Alpha<sub>1</sub>-Adrenoceptor Alterations in Sepsis

Since we had previously found changes in hepatic alpha<sub>1</sub>-adrenoceptors, it was reasonable to suggest that aortic receptors might be similarly altered. Figure 3 shows, using [<sup>125</sup>I]-HEAT as a ligand, a 50% reduction in rat aortic alpha<sub>1</sub>-adrenoceptors during sepsis. The maximum number of binding sites is decreased without a change in affinity (Table III), suggesting fewer ligand recognition sites. These results suggest that the decrease in number in alpha<sub>1</sub>-adrenoceptors could contribute to the observed alterations in signal transduction.

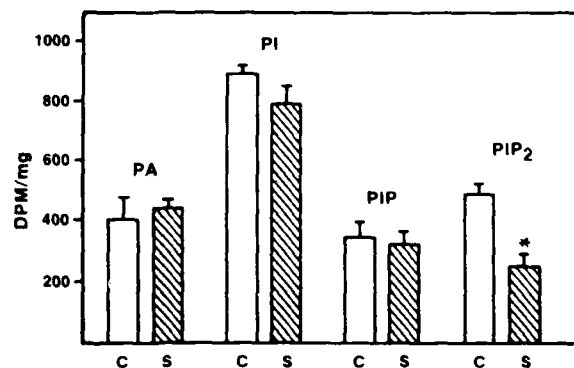


Fig. 2. The effect of intraperitoneal sepsis on basal polyphosphoinositide labeling in rat aorta. Rat aortic rings were incubated with [ $^{32}$ P]-orthophosphate as described in Methods and phospholipids isolated and quantitated. Data represent mean  $\pm$  SEM for six individual determinations. (\* $P < 0.01$ ). C = control; S = septic.

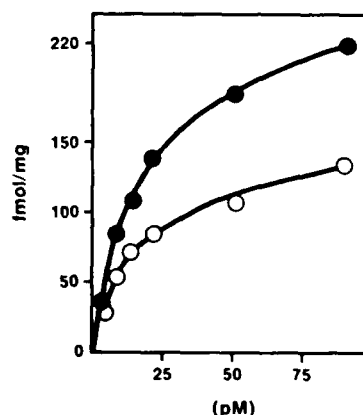


Fig. 3. The effect of intraperitoneal sepsis on rat aortic  $\alpha_1$ -adrenergic receptors. Rat membranes were prepared and incubated with increasing doses of [ $^{125}$ I]-HEAT as described in Methods. Data represent mean of duplicate determinations of specific binding for a typical experiment which has been replicated three times. Computer-derived parameter estimates for sham (closed circles) and septic (open circles) rats are shown in Table III.

TABLE III.  $\alpha_1$ -Adrenoceptor Alteration in Sepsis

Aorta	Control		Septic	
	K <sub>d</sub> (nM)	B <sub>max</sub> (Fmol/mg)	K <sub>d</sub> (nM)	B <sub>max</sub> (Fmol/mg)
Alpha <sub>1</sub> -adrenergic receptors	0.016 $\pm$ 0.003	349 $\pm$ 35	0.013 $\pm$ 0.013	146 $\pm$ 16 <sup>a</sup>

<sup>a</sup> $P < 0.05$  vs. sham operated control rats. Data represent mean  $\pm$  SEM of computer derived estimates for  $N = 3-4$  separate experiments.

## DISCUSSION

Our findings demonstrate that rat aortic alpha<sub>1</sub>-adrenoceptor mediated PI hydrolysis, as well as alpha<sub>1</sub>-adrenoceptors, are significantly altered in rat intraperitoneal sepsis. Studies employing rat aortic preparations *in vitro* have also demonstrated attenuated alpha<sub>1</sub>-adrenoceptor responsiveness [5]; our findings suggest that at least a part of this change in responsiveness could be related to the signal transduction pathway involving alpha<sub>1</sub>-adrenoceptors. Our findings further suggest that defects may reside in the synthesis of the substrate (i.e., PIP<sub>2</sub>) for the nucleotide-activated phospholipase C. We have recently obtained evidence suggesting that these observed biochemical alterations have further consequences. For example, we discovered that NE-induced bidirectional calcium fluxes [37] as well as NE-induced phosphorylation of contractile proteins is similarly decreased in aortas from septic rats [30].

The mechanism of these observations is unknown and will require further investigation. The absence of changes in receptor affinity argues against the presence of a reversibly bound inhibitor. Such a substance would cause a decrease in affinity without changing the number of binding sites. Generalized receptor down-regulation as well seems unlikely since we have measured several other receptor types in this sepsis model (e.g., opiate, serotonergic, hepatic beta adrenergic) which were unchanged (not shown).

It is significant that we were able to verify the alterations in NE-induced PI breakdown by two independent techniques. The first was the measurement of [<sup>3</sup>H]-IP accumulation in the presence of LiCl. The main advantage of this technique is its simplicity. This method has certain disadvantages which include the difficulty in measuring the predominant product (IP<sub>3</sub>) in rat aorta. The inability to measure this metabolite in rat aorta has been seen by others [29]. We elected to use a second, independent method which entails the measurement of [<sup>32</sup>P]-PIP<sub>2</sub> breakdown. Although this technique is more laborious, it gave quite similar results. These two techniques showed that the NE-induced PI hydrolysis was significantly decreased in sepsis.

It is conceivable that an endotoxin-derived molecule could elicit the biochemical changes we have discovered. Endotoxin derived molecules have been shown *in vitro* to activate protein kinase C [31]. It is also known that activation of protein kinase C by phorbol esters induces many of the same changes in PI metabolism in rat aorta as are found in this chronic sepsis model [32]. Thus we showed that phorbol-12,13-dibutyrate as well as phorbol-myristate diacetate attenuated the NE-stimulate PI breakdown in rat aorta [32]. It is also possible that other, unknown, factors contribute to the changes in receptor number and PI metabolism described in this paper.

It is important to note that hepatic alpha<sub>1</sub>-adrenoceptors and vasopressin receptors are also decreased in experimental sepsis and endotoxemia models [17-20]. These findings have been demonstrated to correlate with the attenuation of alpha<sub>1</sub>-adrenoceptor and vasopressin-receptor mediated PIP<sub>2</sub> breakdown, intracellular calcium release and phosphorylase a activation in hepatocytes from septic and endotoxin-treated rats [20,33]. Like aorta, the liver shows a marked insensitivity to alpha<sub>1</sub>-adrenoceptor stimulation in intraperitoneal sepsis [33-36].

In conclusion, we found that rat aortic alpha<sub>1</sub>-adrenoceptor mediated PI hydrolysis, PIP<sub>2</sub> breakdown and receptor numbers were all decreased in intraperitoneal sepsis. We speculate that such alterations could contribute to the previously observed changes in aortic responsivity to NE in sepsis and endotoxemia.

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## REFERENCES

1. Pollack MM, Fields AI, Ruttiman UE: Sequential cardiopulmonary variables of infants and children in septic shock. *Crit Care Med* 12:554-559, 1984.
2. Chernow B, Rainey TG, Lake CR: Endogenous and exogenous catecholamines in critical care medicine. *Crit Care Med* 10:409-416, 1982.
3. Chernow B, Roth BL: The pharmacologic support of the cardiovascular system in septic shock. In Sprung CL, Sibbald W (eds): "New Horizons Focus on Septic Shock." Fullerton: Society of Critical Care Medicine, 1986, Chapter 3.
4. Chernow B, Roth BL: Pharmacologic manipulation of the peripheral vasculature in shock: Clinical and experimental aspects. *Circ Shock* 18:141-155, 1986.
5. Wakabayashi I, Hatake K, Kakishita E, Nagai W: Diminution of contractile response of the aorta from endotoxin-injected rats. *Eur J Pharmacol* 141:117-122, 1987.
6. Pomerantz W, Casey L, Fletcher JR, Ramwell PB: Vascular reactivity in endotoxin. *Adv Shock Res* 7:191-198, 1982.
7. Legan E, Chernow B, Parillo J, Roth BL: Activation of phosphatidylinositol turnover in rat aorta by  $\alpha_1$ -adrenergic receptor stimulation. *Eur J Pharmacol* 110:389-390, 1985.
8. Chiu AT, Pozarh JM, Timmermans PBMWM: Relationship between phosphatidylinositol turnover and  $Ca^{++}$  mobilization induced by  $\alpha_1$ -adrenoceptor stimulation in rat aorta. *J Pharmacol Exp Ther* 240:123-127, 1987.
9. Roth BL, Nakaki T, Chuang D-M, Costa E: Aortic recognition sites for serotonin (5-HT) are coupled to phospholipase C in rat aorta and modulate phosphatidylinositol turnover. *Neuropharmacology* 23:1223-1235, 1984.
10. Suba E, Roth BL: Prostaglandins activate phosphoinositide metabolism in rat aorta. *Eur J Pharmacol* 136:325-332, 1987.
11. Majerus PW, Neufeld EJ, Wilson DB: Production of phosphoinositide-derived messengers. *Cell* 37:701-703, 1984.
12. Berridge MJ: Inositol triphosphate and diacylglycerol as second messengers. *Biochem J* 220:345-360, 1984.
13. Nishizuka Y: The role of protein kinase C in cell surface signal transduction and tumor production. *Nature* 308:693-698, 1984.
14. Suematsu E, Hirata M, Hashimoto T, Kuriyama H: Inositol-1,4,5-triphosphate releases  $Ca^{++}$  from intracellular store sites in skinned single cells of porcine coronary artery. *Biochem Biophys Res Commun* 120:481-485, 1984.
15. Michael RH: Inositol phospholipids and cell surface function. *Biochem Biophys Acta* 415:81-147, 1975.
16. Litten RZ, Suba EA, Roth BL: Effects of a phorbol ester on rat aortic contraction and calcium influx in the presence and absence of BAY k 8644. *Eur J Pharmacol* 144(2):185-193, 1987.
17. McMillan M, Chernow B, Roth BL: Alterations in hepatic  $\alpha_1$ -adrenergic receptors in a rat model of chronic sepsis. *Circ Shock* 19:185-194, 1986.
18. Carcillo JA, Lai J, Venter JC, Roth BL: Alterations in hepatic phospholipase C linked receptors in rat intraperitoneal sepsis. *J Surg Res*, submitted for publication.
19. Roth BL, Spitzer JA: Altered hepatic vasopressin and  $\alpha_1$ -adrenergic receptors after chronic endotoxin infusion. *Am J Physiol* 252:E699-E702, 1987.
20. Spitzer JA, Turco ER, Deaciuc IV, Roth BL: Perturbations of transmembrane signaling mechanisms in acute and chronic endotoxemia. In Schlag G and Heinz R (eds): "First Vienna Shock Forum." *Prog Clin Biol Res* 236A:401-418, 1986.

21. Wichterman K, Baue AI, Chaudry IH: Sepsis and septic shock: A review of laboratory models and a proposal. *J Surg Res* 29:189-201, 1980.
22. Prpic V, Green KC, Blackmore PF, Exton JR: Vasopressin angiotensin II and alpha<sub>1</sub>-adrenergic induced inhibition of Ca<sup>++</sup> transport by rat liver plasma membrane vesicles. *J Biol Chem* 259:1382-1383, 1984.
23. Roth BL, Nakaki T, Chuang D-M, Costa E: Characterization of 5HT<sub>2</sub> receptors coupled to phospholipase C in rat aorta: Modulation of phosphoinositide metabolism by phorbol esters. *J Pharmacol Exp Ther* 238:486-490, 1986.
24. Nakaki T, Roth BL, Chuang D-M, Costa E: Phasic and tonic components in 5HT<sub>2</sub> receptor mediated rat aorta contraction: Participation of Ca<sup>++</sup> channels and phospholipase C. *J Pharmacol Exp Ther* 234:442-446, 1985.
25. Jolles J, Zwiers H, Dekker A, Wirtz KWA, Gispen WH: Corticotropin-(1-24)-tetracosapeptide affects protein phosphorylation and polyphosphoinositide metabolism in rat brain. *Biochem J* 194:283-291, 1981.
26. Munson PB, Rodbard D: Ligand: A versatile computerized approach for characterization of ligand binding systems. *Anal Biochem* 107:220-239, 1980.
27. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248-254, 1976.
28. Roth BL: Modulation of phosphatidylinositol-4,5-bisphosphate hydrolysis in rat aorta by guanine nucleotides, calcium and magnesium. *Life Sci* 41:629-634, 1987.
29. Rapoport, RM: Effects of norepinephrine on contraction and hydrolysis of phosphatidylinositols in rat aorta. *J Pharm Exp Ther* 241:188-194, 1987.
30. Litten RZ, Carcillo JA, Roth BL: Vascular calcium metabolism and protein phosphorylation in rat intraperitoneal sepsis. *Circ Shock* 21:332, 1987.
31. Wightman PD, Raetz CRH: The activation of protein kinase C by biologically active lipid moieties of lipopolysaccharide. *J Biol Chem* 259:10048-10052, 1984.
32. McMillan M, Chernow B, Roth BL: Phorbol esters inhibit alpha<sub>1</sub>-adrenergic receptor stimulated phosphoinositide hydrolysis and contraction in rat aorta: Evidence for a link between vascular contraction and phosphoinositide metabolism. *Biochem Biophys Res Commun* 134:970-974, 1986.
33. Deaciuc IV, Spitzer, JA: Rat liver free cytosolic Ca<sup>2+</sup> glycogen phosphorylase in endotoxemia and sepsis. *Am J Physiol* 251:R984-R995, 1986.
34. Clemens MG, Chaudry IH, McDermott PH: Regulation of glucose production from lactate in experimental sepsis. *Am J Physiol* 244:R794-R800, 1983.
35. Clemens MG, Chaudry IH, Daijneau N, Baue AE: Insulin resistance and depressed gluconeogenic capability during early hyperdynamic sepsis. *J Trauma* 24:701-708, 1984.
36. Carcillo JA, Lai J, Venter JC, Roth BL: Molecular properties of altered alpha<sub>1</sub>-adrenergic receptors in rat intraperitoneal sepsis. *Circ Shock* 21:302, 1987.
37. Litten RZ, Carcillo JA, Roth BL: Alterations in bidirectional transmembrane calcium flux occur without changes in protein kinase C levels in rat aorta during sepsis. *Circ Shock* 25:125-130, 1988.

The experiments described in this paper were performed in adherence to the NIH guidelines for the use of experimental animals.